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Effect of Sampling Site on Retinol, Carotenoid, Tocopherol, and Tocotrienol Concentration of Adipose Tissue of Human Breast with Cancer[1]

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Abstract. The effect of sampling site and closeness of malignant tumor on the retinoid, carotenoid, tocopherol, and tocotrienol concentration of adipose tissue of human breast was studied in 10 cases of breast cancer. The four anatomic quadrants of breast did not differ from each other statistically significantly in relation to adipose tissue concentrations of the vitamins studied. Proximity of malignant tumor did not affect the vitamin concentrations when compared to the more distant sampling sites. Representative sample of breast adipose tissue for vitamin concentration analysis can be obtained from tissue adjacent to the tumor.

Introduction

Studies of the role of nutrition in cancer prevention and treatment have paid a great deal of attention to certain vitamins and vitamin analogues [1]. The most important of these compounds are retinoids, carotenoids, tocopherols, and tocotrienols. Both experimental [2, 3] and epidemiologic studies [4, 5] have demonstrated the chemopreventive properties of retinol and β-carotene. Experimental evidence of the potency of α-tocopherol in cancer prevention is convincing [6, 7], but human data are inconclusive [5, 8]. The purpose of this study was to investigate whether the concentrations of retinoids, carotenoids, tocopherols, and tocotrienols differ in different anatomical locations of breast adipose tissue and whether the concentration in a sample of adipose tissue adjacent to the tumor differs from samples of more distant sites.

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Materials and Methods

Tissue Samples

Human breast adipose tissue samples were obtained from ten ablations performed because of a malignant breast tumor. The samples were removed from the unfrozen tissue block immediately after the operation. Sampling was done by one of the authors (MR). Adipose tissue free of gross blood and debris was taken from each breast at five anatomical locations: upper medial, upper lateral, lower medial, and lower lateral quadrant, and adjacent to the tumor. The adipose samples were transported immediately after sampling in an ice chest to the laboratory where they were divided into parts. These were manually homogenized with a surgical knife and stored in portions of 0.50 g each at – 50 °C until chemical analysis was done.

Retinol and Carotenoid Analysis

The retinol and carotenoid analyses were completed within 4 months of the sampling. The sample (0.25 g) was saponified in ethanolic solution at room temperature. The unsaponifiables were extracted with hexane and after washings transferred to chromatographic eluent. Retinol and carotenoids were determined by normal-phase high-performance liquid chromatography (HPLC): Vydac 101 HSB 5 µm column, a guard column slurry packed with LiChrosorb Si 60 7 µm and a mixture of hexane and 2-propanol (95:5) as the mobile phase. Peak areas of retinol and carotenoids were measured at 325 and 450 nm, respectively. The compounds were identified by comparing their retention times with those of authentic standards. Quantitation of retinol and carotenoids was based on external standard method. Total carotenoids were quantitated as all-*trans*-β-carotene and retinol as all-*trans*-retinol. Calibration curves were linear over the concentration range (16–160 ng/ml) used.

Two identical analytical runs were done for each of the samples, and the final results were calculated as means of the two runs. Repeatability of the method was found to be good with a 5% coefficient of variation [9]. The recoveries of all-*trans*-β-carotene and all-*trans*-retinol was 99 and 86%, respectively [9]. Greater information for the carotenoids was obtained by nonaqueous reversed-phase liquid chromatography used by Nelis and De Leenheer [10].

Tocopherol and Tocotrienol Analysis

The method used here was a microscale modification of the one used for food sample analysis [11]. The tocopherol and tocotrienol analyses were completed within 2 weeks of the sampling. Before analysis, sample (0.25 g) was saponified at room temperature and the unsaponifiables were extracted with n-hexane. The saponification solution contained ascorbic acid (0.1 g), deionized water (4.0 ml), ethanol (10 ml) and potassium hydroxide (0.5 ml of 50% solution). Tocopherols and tocotrienols were separated and quantitated from the washed, concentrated and filtered extracts with a normal-phase HPLC equipped with a fluorescence detector. Identification of tocopherol peaks was accomplished by comparing their retention times with those of the corresponding tocopherol standards. Cereal extracts were used as standards for the identification of tocotrienols. The quantitations were done using external standards.

Two identical analytical runs were made for each sample and the final results were calculated as means of these runs. The recoveries of α-, β-, and γ-tocopherols added to adipose tissue samples (n = 5) were 91, 65, and 60%, respectively. The reproducibility of the tocopherol determinations was good with 5% coefficient of variation. Recoveries of tocotrienols could not be determined because we did not obtain pure tocotrienol standards; however, tocotrienols have been found to behave very similarly to the corresponding tocopherols [11]. δ-Tocopherol was analyzed only qualitatively.

Results

Samples from the four anatomic sampling sites did not differ statistically significantly from each other with respect to any of the absolute levels of vitamins studied. The results were analyzed with the analysis of variance. Therefore, only the mean levels of the four quadrants are compared to that of the tumor-adjacent sample. This is shown in table 1. No differences were observed between the samples from adjacent to the tumor and the samples from the more distant quadrant sites.

Table 1. Tocopherol, tocotrienol, retinol, and carotenoid concentrations of adipose tissue samples taken from human breasts resected for malignancy

Vitamin	Quadrant					Tumor-adjacent			
	mean µg/g tissue	SEM µg/g tissue	mean µg/g fat	SEM µg/g fat	range µg/g tissue	mean µg/g tissue	SEM µg/g tissue	mean µg/g fat	SEM µg/g fat
Carotenoids[1]	3.1	0.4	3.8	0.4	1.0–9.0	3.0	0.6	3.7	0.7
Retinol	2.0	0.2	2.5	0.2	1.0–4.7	2.1	0.3	2.5	0.3
α-Tocopherol	114.3	8.6	126.1	11.2	41.0–234.0	121.3	25.0	146.3	28.4
β-Tocopherol	2.4	0.2	2.8	0.3	0.6–7.8	2.2	0.4	2.5	0.5
γ-Tocopherol	5.1	0.5	5.7	0.6	0.6–12.3	4.7	0.8	5.4	0.9
δ-Tocopherol	trace	–	trace	–		trace	–	trace	–
Total tocopherols	121.8		134.6			128.2		154.2	
α-Tocotrienol	trace	–	trace	–		trace	–	trace	–
β-Tocotrienol	trace	–	trace	–		trace	–	trace	–

The means of 'Quadrant' concentrations are based on 10 cases and adipose tissue samples from four breast sites per case (upper and lower lateral and medial) (n = 40). The means of 'Tumor-adjacent' concentrations are based on the adipose tissue samples taken from adjacent to the tumor from the same 10 cases (n = 10). SEM = Standard error of the mean.

[1] All-*trans*-β-, 15-*cis*-β-, α-carotene, cryptoxanthin, lycopene; quantified by nonaqueous reversed-phase chromatography [9].

Discussion

Studies of retinol and carotenoid concentration of adipose tissue are fewer than those of tocopherol concentration, partly due to the fact that in humans most stored retinol and the highest tissue carotenoid concentrations are found in the liver [12, 13]. Also, previously the role of human adipose tissue in retinol and carotenoid storage has not been viewed as being important. Some animal studies have even reported that β-carotene is absent in adipose tissue [14]. Most of the total body carotenoids in humans are, however, associated with adipose tissue [15]. Since the mass of adipose tissue in humans is much greater than that of the liver, then despite a lower carotenoid concentration, reserves of carotenoids in adipose tissue are much greater than in the liver.

Our results are in accord with the findings of Raica et al. [16] who showed a total carotenoid concentration of 3.9 µg/g tissue and retinol and retinyl ester concentration of 1.46 µg/g tissue.

Tocopherols and tocotrienols are present in almost every diet. The relationship and interaction between oral intake, intestinal absorption, blood level, and tissue concentration of tocopherol is, however, still under investigation. It is known that long-term oral supplementation of tocopherol affects tissue stores more than blood levels [17]. This suggests that the tocopherol composition of adi-

pose tissue varies with the amount of supplementation and, at least to some degree, could change with different dietary intake.

Earlier studies of the tocopherol content of adipose tissue have not presented uniform results. Dju et al. [18] reported total tocopherol concentration of 150 μg/g fat (104 μg/g tissue). Bieri and Poukka Evarts [19] reported α- and γ-tocopherol concentrations ranging from 75 to 629 μg/g tissue and from 32 to 148 μg/g tissue, respectively. However, the number of subjects in their study was very small. Kayden [17] reported a 'normal' concentration of tocopherol to be 262 μg/g fat, based on 4 subjects. This is about twice as much as in the present study. Tissue storage may account for some of these differences. Storage of samples at −20 °C for 4 weeks resulted in a 20% loss of the tocopherol content [20]. In the present study, the tissue samples were stored at −50 °C and it is not known how the storage of up to 2 weeks affected the vitamin concentrations. Some minor loss did probably occur.

Since tissue concentration of vitamins depends on several factors including age of the subjects [18], nutritional and other intake of the vitamins [17], sampling site [18], storage time [20], and analytical method employed, comparisons between different studies may not be useful. Each investigation should therefore include its own control or comparison group and samples.

The results of this study indicate that proximity to a malignant tumor does not affect the retinol, carotenoid, tocopherol, and tocotrienol composition of adipose tissue when compared to more distant sampling sites. A representative sample of adipose tissue can be obtained in a breast lumpectomy of practically any kind, which makes comparisons of cases with different type of breast tumors possible. We obtained no adipose tissue from other parts of the body and thus it is not possible to conclude whether our results are applicable to the situation in different parts of body and in different kinds of fat.

References

1 Committee on Diet, Nutrition, and Cancer: Assembly of Life Sciences, National Research Council. Washington, National Academy Press, 1982.

2 Lupulescu A: Inhibition of DNA synthesis and neoplastic cell growth by vitamin A (retinol). JNCI 1986;77:149–156.

3 Fraker LD, Halter SA, Forber JT: Growth inhibition by retinol of a human breast carcinoma cell line in vitro and in athymic mice. Cancer Res 1984;44:5757–5763.

4 Sawicki J, Ostrowski J, Swietochowska B, et al: Vitamin A (retinol) level in colon and lung cancer patient sera. Neoplasma 1985;32:225–227.

5 Shekelle RB, Lepper M, Liu S, Maliza C, Raynor WJ Jr, Rossof AH: Dietary vitamin A and risk of cancer in the Western Electric Study. Lancet 1981;ii:1185–1190.

6 Cook MG, McNamara P: Effect of dietary E on dimethylhydrazine-induced colonic tumors in mice. Cancer Res 1980;40:1329–1331.

7 Rama BN, Prasad KN: Effects of *dl*-alpha-tocopherol succinate in combination with sodium butyrate and cAMP stimulating agent on neuroblastoma cells in culture. Int J Cancer 1984;34:863–867.

8 Wald NJ, Boreham J, Hayward JL, Bulbrook RD: Plasma retinol, beta-carotene and vitamin E levels in relation to the future risk of breast cancer. Br J Cancer 1984;49:321–324.

9 Heinonen M: HPLC-determination of retinol and carotenoids in adipose tissue (in Finnish, English abstract), EKT-series 722. Helsinki, University of Helsinki, 1986.

10 Nelis MJ, De Leenheer AP: Isocratic non-aqueous reversed-phase liquid chromatography of carotenoids. Anal Chem 1983;55:270–275.

11 Piironen V, Varo P, Syväoja E-L, Salminen K, Koivistoinen P: High-performance liquid chro-

matographic determination of tocopherols and tocotrienols and its application to diets and plasma of Finnish men. I. Analytical method. Int J Vitam Nutr Res 1984;53:35–40.
12 Smith BM, Malthus EM: Vitamin A content of human liver from autopsies in New Zealand. Br J Nutr 1962;16:213–218.
13 Hoppner K, Phillips WEJ, Erdody P, Murray TK, Perrin DE: Vitamin A reserves of Canadians. Can Med Assoc J 1969;13:84–86.
14 Shapiro SS, Mott DJ, Machlin LJ: Kinetic characteristics of beta-carotene uptake and depletion in rat tissue. J Nutr 1984;114:1924–1933.
15 Olson JA: Serum levels of vitamin A and carotenoids as reflectors of nutritional status. JNCI 1984;73:1439–1444.
16 Raica N Jr, Scott J, Lowry L, Sauberlich HE: Vitamin A concentration in human tissues collected from five areas in the United States. Am J Clin Nutr 1972;25:291–296.
17 Kayden HJ: Tocopherol content of adipose tissue from vitamin E-deficient humans. Ciba Found Symp 1983;101:70–85.
18 Dju MY, Mason KE, Filer LJ: Vitamin E (tocopherol) in human tissues from birth to old age. Am J Clin Nutr 1958;6:50–60.
19 Bieri JG, Poukka Evarts R: Tocopherols and polyunsaturated fatty acids in human tissues. Am J Clin Nutr 1975;28:717–720.
20 Dju MY, Filer LJ, Mason KE: Effect of storage on tissue tocopherols. Am J Clin Nutr 1958;6:61–64.

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